CA 19-9 (Cancer Antigen) Elisa

CAT NO	DESCRIPTION	PACK SIZE
EIA1991	CA 19-9 Elisa	96 Tests

Intended Use:

CA 19-9 Elisa is intended to be used for the quantitative determination of CA 19-9 in Human serum. This reagent is for In vitro Diagnostic use only.

Summary and Principle:

CA 19-9 is a mucin type SLA glycoprotein and they have been associated with being circulatory antigens of gastrointestinal cancer. It is commonly used to differentiate between pancreatic cancer with other non-cancerous conditions as well as to monitor response to pancreatic cancer treatment and to check for recurrence. It may be requested along with other tumour markers such as CEA. It has been found to increase in other malignancies such as colorectal, gastric and hepatic carcinomas. It is also suggestive of gall bladder neoplasms when elevated along with CEA. A persistently high CA 19-9 is suggestive of progressive malignant disease and poor therapeutic response. A decrease in CA 19-9 is indicative of good prognosis.

Reagent Composition:

COMPONENT	SIZE	DESCRIPTION
Microwell Plate	1x96 wells (12x8 well plate)	Each microwell is coated with monoclonal anti CA 19-9 antibodies. The microwells can be broken and used separately. Place unused wells or strips in the provided plastic sealable bag together with the desiccant and store at 2-8°C. Once open the wells are stable until expiry date at 2-8°C.
CA 19-9 Calibrators	6x1.0ml	6 vials containing CA 19-9 at concentrations of 0.0, 15, 30, 60, 120 and 240 U/ml made up in a human serum matrix. CONCENTRATIONS GIVEN IN THE IFU ARE SUBJECT TO CHANGE. Ready to use. Once open stable until expiry date at 2-8°C.
Assay Buffer	1x12ml	1 vial containing 12ml of Assay Buffer. Once open, stable until expiry date at 2-8°C.
Enzymatic Conjugate	1x12ml	1 vial containing 12ml of HRP labelled monoclonal Anti CA 19-9 antibodies in Buffered saline. Once open, until expiry date at 2-8°C.
Wash Buffer Concentrate (50X)	1x15ml	PBS-Tween at pH 7.4. 50X concentrate. The concentrate must be diluted with 735ml of distilled water before use. Once diluted it is stable at room temperature for two months.
Substrate Solution	1x12ml	Mixture of TMB and Hydrogen Peroxide solution. Ready to use. Once open, stable until expiry date at 2-8°C.
Stop Solution	1x12ml	Diluted Sulfuric acid solution (1M) Ready to use. Once open, stable until expiry date at 2-8°C.

Plastic Sealable bag, IFU and plate covers.

Materials required but not provided:

Distilled water, Vortex mixer, Micropipettes, Incubator, Microplate Reader and Microplate washer.

Specimen Collection:

Serum should be prepared from whole blood specimen obtained by acceptable medical techniques. Avoid grossly haemolytic, lipaemic or turbid samples. Plasma samples collected in tubes containing EDTA, heparin or oxalate may interfere with the test procedures and should be avoided. Specimen should be capped and may be stored up to 48 hours at 2-8°C, prior to assaying. Specimens held for a longer time can frozen at -20°C. Thawed samples must be mixed prior to testing.

Storage and Stability:

The contents of the kit will remain stable up to expiry date when stored at 2-8°C. Do not freeze. Keep all components tightly capped and without any contamination. Place unused wells in zip-lock bag provided and return to 2-8°C, under which conditions the wells will remain stable until the labelled expiry date. Seal and return all the other unused reagents to 2-8°C, under which conditions the stability will be retained until the labelled expiry date.

Procedure:

Reagent preparation:

1. Bring all reagents to room temperature (18-22°C) prior to use.
2. Dilute the wash buffer concentrate with 735ml of Distilled water (yielding a total volume of 750ml). Once diluted the wash solution is stable for 2 months at room temperature. Mix well before use.

STEP 1

Preparation: Remove the number of wells required and number each well for the assay series.

STEP 2

Addition of Samples and calibrators: Add 50 µl of Calibrators and Samples to each well.

STEP 3

Addition of Assay Buffer: Add 100 µl of the Assay Buffer to each well. Shake the plate for 5 seconds to ensure that the added components are well mixed.

STEP 4

Incubation: Cover the plate with the plate cover and incubate for 60 minutes at 37°C

STEP 5

Washing: At the end of the incubation period, remove and discard the plate cover. Wash each well 5 times with diluted washing buffer of 350 µl. After the final washing cycle, turn down the plate onto a blotting paper or a clean towel and tap it to remove any residual buffer.

STEP 6

Addition of Enzyme Conjugate: Add 100 µl of the Enzyme Conjugate to each well. Shake the plate for 5 seconds to ensure that the added components are well mixed.

STEP 7

Incubation: Cover the plate with the plate cover and incubate for 60 minutes at 37°C.

STEP 8

Washing: At the end of the incubation period, remove and discard the plate cover. Wash each well 5 times with diluted washing buffer of 350 µl. After the final washing cycle, turn down the plate onto a blotting paper or a clean towel and tap it to remove any residual buffer.

STEP 9

Addition of the Substrate: Add 100 µl of Substrate Solution to each well. Mix gently for 10 seconds.

STEP 10

Incubation: Cover the plate with the plate cover and incubate for 20 minutes at room temperature. Ensure that the incubation is done in the dark.

STEP 11

Stopping the Reaction: Add 100 µl of the Stop solution into each well and mix gently. Shake the plate to mix till the solution changes to yellow from blue.

STEP 12

Measurement: Read the absorbance of the wells at 450/630nm using a microplate reader within 15 minutes of adding the Stop Solution. Note down the absorbances.

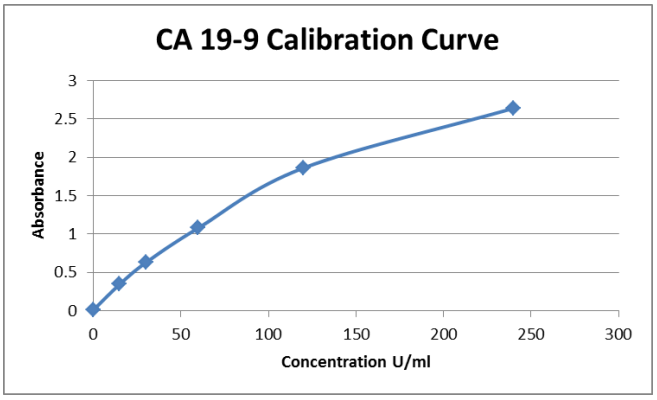
Note: The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings. It is recommended that no more than 32 wells are used for each assay run if manual pipetting is used since pipetting of all calibrators, specimens, controls should be completed within 3 minutes. A full plate of 96 wells may be used if automated pipetting is available. Duplication of calibrators and specimens although not required is recommended.

Calculation of results:

- Record the absorbances obtained from the microplate reader. Ensure that mean absorbances are calculated for duplicate measurements.
- Plot the absorbance in Y axis and Concentration in U/ml in X axis.
- Draw a point to point curve through the plotted points on a linear graph paper.
- To determine the concentration of an unknown sample, locate the absorbance of the sample on the Y axis and find the intersecting point on the curve. Read the concentration from the X axis by dropping a line from the intersecting point of the absorbance on the curve.

Example:

ID	ABSORBANCE OF CALIBRATORS	CONCENTRATION OF CALIBRATORS
CAL A	0.078	0.0 U/ml
CAL B	0.620	15.0 U/ml
CAL C	1.009	30.0 U/ml
CAL D	1.562	60.0 U/ml
CAL E	2.182	120.0 U/ml
CAL F	2.742	240.0 U/ml



This calibration curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain their own curve and data.

Expected Values:

In healthy individuals CA 19-9 values are generally below 35 U/ml.

Performance Characteristics:

1. **Intra assay Precision:**

Panel	Data no.	Mean	SD	CV%
1	24	11.70	0.885	7.56%
2	24	33.33	1.540	4.62%

2. **Inter assay Precision:**

Panel	Data no.	Mean	SD	CV%
1	14	11.76	1.098	9.81%
2	14	33.15	2.160	6.52%

3. **Sensitivity:**

The minimum detectable concentration of CA 19-9 by this assay was found to be 5 U/ml.

Linearity:

A patient serum was serially diluted with 0 U/ml standard in a linearity study. The average recovery was 102.7%

Sample A			
Dilution	Expected	Observed	% Recovery
Sample undiluted	192.43 U/ml	192.43 U/ml	
2x	96.22 U/ml	98.11 U/ml	102.0 %
4x	48.10 U/ml	50.01 U/ml	104.0 %
8x	24.05 U/ml	25.98 U/ml	108.0 %
16x	12.02 U/ml	13.11 U/ml	109.1 %
Average Recovery: 105.8%			

Sample B			
Dilution	Expected	Observed	% Recovery
Sample undiluted	220.77 U/ml	220.77 U/ml	
2x	110.39 U/ml	106.31 U/ml	96.3 %
4x	55.19 U/ml	56.03 U/ml	101.5 %
8x	27.60 U/ml	26.92 U/ml	97.5 %
16x	13.80 U/ml	14.25 U/ml	103.3 %
Average Recovery: 99.7%			

Method Comparison:

Method comparison between this assay and a commercially available assay yielded the following data:
N=48, Correlation Coeff: 0.966, Slope: 0.908, Intercept: 2.32
Mean values: This assay: 36.10 U/ml and comparator Abbott: 33.18 U/ml

Cross Reactivity:

The following materials were tested for cross reactivity and the results are as follows:

Antigens	Concentration	Equivalent CA 19-9	% Cross reactivity
HCG	400 IU/ml	0.0 U/ml	0.0 %
PAP	1000 ng/ml	0.0 U/ml	0.0 %
PSA	1000 ng/ml	0.0 U/ml	0.0 %
AFP	1000 ng/ml	0.0 U/ml	0.0 %

No High dose hook effect was observed up to 40,000 U/ml CA 19-9 in this assay.

References:

1. Glenn., J., Steinberg W.M Kurtzman, S.H et al. Evaluation of the utility of a radioimmunoassay for serum CA 19-9 level in patients before and after treatment of carcinoma of the pancreas. J.Clin oncol 1988:6-462-8.

2. Wang, T.H Lin J.W., Chen D.S et al. non-invasive diagnosis of advanced pancreatic cancer by real time ultrasonography. Pancrease 1986; 1:219-23.

REF	Catalog number		Temperature limitation
	Consult instructions for use	LOT	Batch code
IVD	*In vitro* diagnostic medical device		Use by
	Manufacturer		

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